Miltenyi Biotec

Lamina Propria Dissociation Kit
mouse

Order no. 130-097-410

Contents

1. Description

Components 5 vials, containing:

2 vials of Enzyme D (lyophilized powder)
1 vial of Enzyme R (lyophilized powder)
1 vial of Enzyme A (lyophilized powder)
1 vial of Buffer A

Size For 50 digestions.

The specified number of digestions is valid when digesting small intestine tissue up to a weight of 1 g following the protocol in chapter 2.2.

Storage Upon arrival immediately store all components at 2–8 °C. Reconstitute all components before the date indicated on the box label. For information about reconstitution and storage after reconstitution of the lyophilized components refer to chapter 2.1.

1.1 Principle of the Lamina Propria Dissociation Kit

Lamina propria tissue can be dissociated to single-cell suspensions by combining mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins which maintain the structural integrity of tissues.
In a first step, the intraepithelial lymphocytes (IELs) are disrupted from the mucosa by shaking the tissue in a predigestion solution.
Then, the lamina propria tissue is further treated enzymatically and mechanically dissociated into a single-cell suspension by using the gentleMACS™ Dissociators.
Cells should be processed immediately for downstream applications, such as MACS® MicroBead separations, cellular or molecular analysis.

1.2 Background information

The Lamina Propria Dissociation Kit, mouse has been developed for the gentle, rapid, and efficient generation of single-cell suspensions from mouse small intestine lamina propria tissue. It is optimized for a high yield of small intestine lamina propria lymphocytes, while preserving cell surface epitopes. Isolated cells can be analyzed directly or subjected to magnetic cell separation using MACS Technology to obtain specific cell subsets.

Dissociated cells can be analyzed *in vitro* or subpopulations of cells can be isolated using MACS Technology.

1.3 Applications

- Dissociation of mouse lamina propria tissue derived from 6–10 week old C57BL/6 or Balb/c mice into single-cell suspensions for subsequent cell separations using MACS Technology.
- Phenotyping or enumeration of lamina propria cell populations by flow cytometry.

1.4 Reagent and instrument requirements

- (Sterile) 1× Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} containing 10 mM HEPES, in the following referred to as HBSS (w/o). Store at room temperature.
- (Sterile) 1× HBSS with Ca^{2+} and Mg^{2+} containing 10 mM HEPES, in the following referred to as HBSS (w). Store at room temperature.
- (Sterile) Predigestion solution: prepare 1× HBSS (w/o) containing 5 mM EDTA, 5% fetal bovine serum (FBS), 1 mM DTT freshly before each digestion. Store at room temperature.
  ▲ **Note:** Per digestion a volume of 40 mL of the predigestion solution is required.
- (Sterile) Digestion solution: prepare 1× HBSS (w) containing 5% FBS freshly before each digestion. Store at room temperature.
  ▲ **Note:** Per digestion a volume of 2.5 mL of the digestion solution is required.
- (Sterile) PB buffer: prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5 % bovine serum albumin (BSA). Keep buffer cold (2–8 °C).
  ▲ **Note:** BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or FBS.
- MACS SmartStrainers, 100 µm (# 130-098-463)
- MACSmix™ Tube Rotator (# 130-090-753) in combination with an incubator at 37 °C.
- gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) ART® 1000 REACH™ pipet tips (Molecular BioProducts, Inc.) for removal of dissociated material from the closed C Tubes.

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2. Protocol

▲ For details on the use of the gentleMACS Dissociators, refer to the respective user manuals.

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ Volumes given below are for up to 1 g of starting tissue material, which is approximately the weight of a small intestine derived from one 6–10 week old C57BL/6 or Balb/c mouse. When working with less than 1 g, use the same volumes as indicated, but do not use less than 0.8 g for each digestion.

▲ Operate MACSmix Tube Rotator with continuous rotation at a speed of approximately 12 rpm.

2.1 Reagent preparation

1. Prepare Enzyme D by reconstitution of the lyophilized powder in each vial with 3 mL of sterile HBSS (w). Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution. For cell culture experiments subsequent to tissue dissociation, Enzyme D should be sterile filtered prior to aliquoting.
2. Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 2.7 mL sterile HBSS (w). Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

   ▲ **Note:** Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume!
3. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of Buffer A supplied with the kit. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution.

2.2 Lamina propria dissociation protocol

1. Remove intestine from a 6–10 week old C57BL/6 or Balb/c mouse and place it in HBSS (w/o) in a Petri dish.
2. Clear the intestine of feces by holding it with forceps and flushing with HBSS (w/o) using a syringe.
3. Remove residual fat tissue and Peyer's patches.
4. Cut the intestine first longitudinally and then laterally into pieces of approximately 0.5 cm length.
5. Transfer the tissue pieces into a 50 mL tube containing 20 mL of predigestion solution.
6. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
7. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

   ▲ **Note:** Flow through contains intraepithelial lymphocytes (IELs). If IEL isolation is desired store flow-through on ice.
8. Transfer the lamina propria tissue pieces into a new 50 mL tube containing 20 mL of fresh predigestion solution.
9. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
10. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

    ▲ **Note:** If IEL isolation is desired, combine the flow through, which contains IELs, with flow through from step 7. Store the cell suspension for at least 10 minutes on ice and transfer the supernatant that contains the cells then into a new tube.
11. Transfer the lamina propria tissue pieces into a new 50 mL tube containing 20 mL of HBSS (w/o).
12. Incubate the sample for 20 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.

    ▲ **Note:** In the meantime: Transfer 2.35 mL of digestion solution into a gentleMACS C Tube and pre-heat at 37 °C for 15 minutes.
13. Mix well for 10 seconds using a vortex mixer and apply the sample onto a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

    ▲ **Note:** If IEL isolation is desired, combine the flow through, which contains IELs, with supernatant from step 10. Centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely and resuspend IELs with appropriate buffer to the required volume for further applications.
14. Prepare enzyme mix by adding 100 µL of Enzyme D, 50 µL of Enzyme R, and 12.5 µL of Enzyme A into a gentleMACS C Tube containing the pre-heated 2.35 mL of digestion solution and mix gently.
15. Transfer the intestine tissue into the gentleMACS C Tube containing the enzyme mix and close C Tube tightly.
16. (Optional) If using the heating function of the gentleMACS Octo Dissociator with Heaters run program **37C_m_LPDK_1** and continue with step 20.
17. Incubate the sample for 30 minutes at 37 °C under continuous rotation using the MACSmix Tube Rotator.
18. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.

    ▲ **Note:** Close C Tube tightly beyond the first resistance.

    ▲ **Note:** It has to be ensured that the sample material is located in the area of the rotor/stator.
19. Run the gentleMACS Program **m_intestine_01**.
20. After termination of the program, detach C Tube from the gentleMACS Dissociator and perform a short spin up to 300×g to collect the sample at the bottom of the tube.
21. Resuspend sample, add 5 mL of PB buffer, and apply the cell suspension to a MACS SmartStrainer, 100 µm, placed on a 50 mL tube.

    ▲ **Note:** Dissociated tissue can be removed from the closed C Tube by pipetting through the septum-sealed opening in the center of the cap of the C Tube. Use ART 1000 REACH 1000 µL pipette tips.
22. Wash MACS SmartStrainer, 100 µm, with 10 mL of PB buffer.
23. Discard the MACS SmartStrainer, 100 µm, and centrifuge cell suspension at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
24. Resuspend lamina propria lymphocytes with an appropriate buffer to the required volume for further applications, for example, resuspend cells in PB buffer for magnetic cell separation or flow cytometry.
25. Cells should be processed immediately for further applications.
26. (Optional) To remove erythrocytes or dead cells, perform a density gradient centrifugation step.

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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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